APPENDIX C

ABSTRACT OF PROTOCOL

Autologous bone marrow transplantation (ABMT) is an effective way of treating AML. A proportion of the patient's marrow is removed during remission of the malignancy and cryopreserved. The patient is then given supralethal chemotherapy and/or radiotherapy and rescued from the associated marrow destruction by reinfusion of the stored cells. The two main limitations of the approach are: (1) the high risk of relapse, which may occur because the reinfused marrow is contaminated with malignant cells, and (2) the long delay before the marrow reconstitutes the patient with a consequent high risk of morbidity and mortality from infection and hemorrhage. The current proposal aims to transduce marker genes into harvested marrow to allow investigation of therapeutic strategies aimed at overcoming these problems.

If the patients have a disease such as leukemia in which malignant cells may contaminate the marrow, these cells may be marked by the gene transfer. Relapse with cells containing marker genes will then establish whether or not relapse originates within the patient or at least partly within the marrow cells harvested at the time of clinical remission. Should this latter explanation be correct, it would justify the procedure of marrow purging at harvest to remove malignant cells. At present, this procedure is of unproven benefit, but damages the marrow and retards engraftment, correspondingly increasing morbidity and mortality. Analysis of insertion sites will allow determination of clonality of relapse. The outcome of these investigations would affect future treatment strategies.

Since marrow for ABMT is generally obtained when patients are regenerating their marrow after intensive chemotherapy aimed at remission induction, it is anticipated that a proportion of pluripotent/stem cells will be in cycle and may therefore be successfully transduced before cryopreservation and subsequent reinfusion. Marker gene insertion will be determined by the appearance of G418 resistance amongst cultured progenitor cells in the engrafted marrow and by detection of vector sequences by PCR or Southern blot analysis of DNA from circulating cells of different lineages. We will determine what endogenous and exogenous stimuli, such as infection, chemotherapy, and recombinant growth factors, can augment the proliferation of this engrafted marrow. This information will help to determine whether autologous marrow genuinely re-populates the patient or whether it provides temporary replenishment of committed progenitor cells whilst surviving host stem cells gradually repopulate. In the future it may also be used to indicate what stimuli should be given before the marrow harvest to ensure maximum yield of pluripotent progenitors and which stimuli should be used immediately following the transplant to induce more rapid engraftment. With the additional knowledge obtained, better clinical protocols can be established.